

ZEITSCHRIFT FÜR NATURFORSCHUNG

SECTION C

A EUROPEAN JOURNAL OF
BIOSCIENCES

Council

E. BÜNNING, Tübingen
A. BUTENANDT, München
M. EIGEN, Göttingen

Editorial Board

A. HAGER, Tübingen
K. HAHNBROCK, Köln
W. HASSELBACH, Heidelberg
P. KARLSON, Marburg
F. KAUDEWITZ, München
J. KLEIN, Tübingen
J. ST. SCHELL, Köln
E. WECKER, Würzburg

Advisory Editorial Board

N. AMRHEIN, Bochum	G. ISENBERG, Köln	J. SEELIG, Basel
B. A. ASKONAS, London	R. JAENICKE, Regensburg	H. SIMON, München
W. BARZ, Münster	V. TER MEULEN, Würzburg	W. STEGLICH, Bonn
P. BÖGER, Konstanz	G. F. MEYER, Tübingen	H. STIEVE, Aachen
G. BORNKAMM, Freiburg	M. RAJEWSKY, Essen	J. SUKO, Wien
D. BÜCKMANN, Ulm	H. SCHIMASSEK, Heidelberg	A. TREBST, Bochum
K. G. GÖTZ, Tübingen	D. SCHULTE-FROHLINDE, Mühlheim/R.	G. WEISSENBOCK, Köln
G. GOTTSCHALK, Göttingen	G. SCHULZ, Freiburg	G. WICK, Innsbruck
P. GRUSS, Heidelberg	F. F. SEELIG, Tübingen	V. ZIMMERMANN, Würzburg

EDITED IN COLLABORATION

WITH THE INSTITUTES OF THE MAX-PLANCK-GESELLSCHAFT

Volume 41 c

1986

VERLAG DER ZEITSCHRIFT FÜR NATURFORSCHUNG
TÜBINGEN

Contents

Contents of Number 1/2

Original Communications

- Identification of (*R*)-Vicianin in *Davallia trichomanoides* Blume
P. A. LIZOTTE and J. E. POULTON 5
- Differential Regulation of Two Genes Controlling the Biosynthesis of Isovitexin 7-O-Galactoside in *Silene* Plants
J. M. STEYNS and J. v. BREDERODE 9
- Biosynthetic Capacity of *Stachys* Seedlings for Verbascoside and Related Caffeoyl Derivatives
C. ANDARY and R. K. IBRAHIM 18
- Isolation and Separation of Epidermal and Mesophyll Protoplasts from Rye Primary Leaves – Tissue-Specific Characteristics of Secondary Phenolic Product Accumulation
M. SCHULZ and G. WEISSENBOCK 22
- Partial Purification and Some Properties of 1-Sinapoylglucose: Choline Sinapoyltransferase ("Sinapine Synthase") from Seeds of *Raphanus sativus* L. and *Sinapis alba* L.
W. GRÄWE and D. STRACK 28
- Biosynthesis of the Furanoacetylene Phytoalexin Weyerone in *Vicia faba*
N. A. AL-DOURI and P. M. DEWICK 34
- Host-Pathogen Interactions. XXX. Characterization of Elicitors of Phytoalexin Accumulation in Soybean Released from Soybean Cell Walls by Endopolygalacturonic Acid Lyase
K. R. DAVIS, A. G. DARVILL, P. ALBERSHEIM, and A. DELL 39
- Inhibition of Phenylalanine Ammonia-Lyase *in vitro* and *in vivo* by (1-Amino-2-phenylethyl)phosphonic Acid, the Phosphonic Analogue of Phenylalanine
B. LABER, H.-H. KILTZ, and N. AMRHEIN 49
- Flavin Nucleotide-Dependent 3-Hydroxylation of 4-Hydroxyphenylpropanoid Carboxylic Acids by Particulate Preparations from Potato Tubers
J. M. BONIWELL and V. S. BUTT 56
- Use of Immunotitration to Demonstrate Phytochrome-Mediated Synthesis *de novo* of Chalcone Synthase and Phenylalanine Ammonia Lyase in Mustard Seedling Cotyledons
R. BRÖDENFELDT and H. MOHR 61
- Tyrosine Biosynthesis in *Sorghum bicolor*: Isolation and Regulatory Properties of Arogenate Dehydrogenase
J. A. CONNELLY and E. E. CONN 69
- Tyrosine Biosynthesis in *Sorghum bicolor*: Characteristics of Prephenate Aminotransferase
D. L. SIEHL, J. A. CONNELLY, and E. E. CONN 79
- Flavonoids and Terpenoids from the Exudates of Some *Baccharis* Species
E. WOLLENWEBER, I. SCHÖBER, P. DOSTAL, D. HRADETZKY, F. J. ARRIAGA-GINER, and G. YATSKIEVYCH 87
- Transformation-Related Cellular Protein p53: Increased Level in Untransformed Rat Cells Following Treatment with the Tumorpromoter, Tetradecanoylphorbol-Acetate
M. HEBEL, G. BRANDNER, H. K. HOCHKEPPEL, and D. G. BRAUN 94
- Glycosphingolipid Analysis of Human Myeloid Leukemias (In German)
B. KNIEP and P. F. MÜHLRADT 100
- Properties of Vinorine Synthase – the *Rauwolfia* Enzyme Involved in the Formation of the Ajmaline Skeleton
A. PFITZNER, L. POLZ, and J. STÖCKIGT 103
- High Performance Liquid Chromatography Coupled with Radioactivity Detection: A Powerful Tool for Determining Drug Metabolite Profiles in Biological Fluids
K.-O. VOLLMER, W. KLEMISCH, and A. v. HODENBERG 115
- Partial Purification and Characterization of S-Adenosyl-L-Methionine:Norreticuline N-Methyltransferases from *Berberis* Cell Suspension Cultures
CHI-KIT WAT, P. STEFFENS, and M. H. ZENK 126

Biosynthesis of 4-Formyl-4-imidazoline-2-on, the Heterocyclic Base of Nikkomycin X R.-M. SCHMIDT, H. PAPE, and M. JUNACK	135	the White-Rot Fungus, <i>Phanerochaete chrysosporium</i> M. ARJMAND and H. SANDERMANN JR.	206
Metabolism of the Plant Growth Regulator (<i>E</i>)-[³ H]2-Ethylhex-2-enoic Acid in <i>Hordeum vulgare</i> B. SCHNEIDER, H.-R. SCHÜTTE, and A. PREISS	141	Further Studies on the Biosynthesis of Granaticin XIAN-GUO HE, CHIU-CHIN CHANG, CHING-JER CHANG, J. C. VEDERAS, A. G. MCINNES, J. A. WALTER, and H. G. FLOSS	215
Metabolism and Degradation of Nicotinic Acid in Parsley (<i>Petroselinum hortense</i>) Cell Suspension Cultures and Seedlings L. SCHWENEN, D. KOMOSSA, and W. BARZ	148	Minimal Time Requirement for Lasting Elicitor Effects in Cultured Parsley Cells H. STRASSER and U. MATERN	222
Basal Blotches and Blotchlessness of Poppy Flowers: A Chemogenetic Characterization (In German) H. BÖHM	158	Elicitor-Stimulated Furanocoumarin Biosynthesis in Cultured Parsley-Cells: S-Adenosyl-L-Methionine: Bergaptol and S-Adenosyl-L-Methionine: Xanthotoxol O-Methyltransferases K. D. HAUFFE, K. HAHLBROCK, and D. SCHEEL	228
Chemical Investigations of Tropical Medicinal Plants, XXI. Long Chain Alkyl Esters of Ferulic and <i>p</i> -Coumaric Acid from <i>Bauhinia manca</i> H. ACHENBACH, M. STÖCKER, and M. A. CONSTENLA	164	The Topology of the Plastoquinone and Herbicide Binding Peptides of Photosystem II in the Thylakoid Membrane A. TREBST	240
<i>Cyanophora paradoxa</i> : Fatty Acids and Fatty Acid Synthesis <i>in vitro</i> H. KLEINIG, P. BEYER, C. SCHUBERT, B. LIEDVOGEL, and F. LÜTKE-BRINKHAUS	169		
Degradation of NAD(H) by Endogenous Enzymes of Yeasts and Clostridia H.-J. SCHUETZ and H. SIMON	172		
Genetic and Biochemical Studies on the Conversion of Dihydroflavonols to Flavonols in Flowers of <i>Petunia hybrida</i> G. FORKMANN, P. DE VLAMING, R. SPRIBILLE, H. WIERING, and A. W. SCHRAM	179		
Biosynthesis of Acridone Alkaloids. A Cell-free System from <i>Ruta graveolens</i> Cell Suspension Cultures A. BAUMERT, G. SCHNEIDER, and D. GRÖGER	187		
Phytoalexin Production by Isolated Soybean Protoplasts H. MIETH, V. SPETH, and J. EBEL	193		
Composition of Lipopolysaccharides from Various Strains of <i>Rhodocrobium vannielii</i> O. HOLST, J. WECKESSER, B. RIETH, and C. S. DOW	202		
Plant Biochemistry of Xenobiotics. Mineralization of Chloroaniline/Lignin Metabolites from Wheat by			

Contents of Number 3

Original Communications

Biosynthesis of Daphnetin in <i>Daphne mezereum</i> L. ST. A. BROWN	247
Monogalloylhamamelose from <i>Hamamelis virginiana</i> (In German) G. SCHILLING and A. KELLER	253
Activation of Streptolysin S <i>in vitro</i> by Oligonucleotides A. TAKETO and Y. TAKETO	258
Optimization of Conditions for Accurate Phosphate and Total Phosphorus Assay on Lipid Samples, in Conjunction with Thin-Layer Chromatography V. M. KAPOULAS and G. TH. TSANGARIS	263
Effect of the CO ₂ -Concentration during Growth on the Oxygen Evolution Pattern under Flash Light in <i>Chlorella</i> Y. SHIRAIWA and G. H. SCHMID	269

A Large Chloroplast Thioredoxin <i>f</i> Found in Green Algae P. LANGLOTZ, W. WAGNER, and H. FOLLMANN	275	<i>Notes</i>	
Chlorophyll Photobleaching in Pigment-Protein Complexes R. CARPENTIER, R. M. LEBLANC, and G. BELLE-MARE	284	Influence of Temperature on the Transport of Ascorbate across Artificial Membranes as Studied by the Spin Label Technique W. LOHMANN, P. Z. TIAN, and D. HOLZ	348
Diurnal Changes of Fructose-6-phosphate,2-kinase and Fructose-2,6-bis-phosphatase Activities in Spinach Leaves M. STITT, G. MIESKES, H.-D. SÖLING, H. GROSSE, and H. W. HELDT	291	The Influence of Spin Label on the Transport of Ascorbate across Artificial Membranes W. LOHMANN, P. Z. TIAN, and D. HOLZ	351
Molecular Mechanics Investigation on Conformational Flexibility of 14 β Steroids in Drug-Receptor Interactions M. BOHL and M. WUNDERWALD	297	Effect of Magnetic Field on Ascorbic Acid Oxidase Activity, I V. S. GHOLE, P. S. DAMLE, and W. H.-P. THIEMANN	355
Alterations in the Activities of Rabbit Erythrocyte Membrane-Bound Enzymes Induced by Cholesterol Enrichment and Depletion Procedures E. KAMBER and L. KOPEIKINA-TSIBOUKIDOU	301	Oxygen Incorporation in Cleavage of ¹⁸ O-Labeled 13-Hydroperoxylinoleyl Alcohol into 12-Hydroxy-(3 <i>Z</i>)-dodecenal in Tea Chloroplasts A. HATANAKA, T. KAJIWARA, J. SEKIYA, and H. TOYOTA	359
Stimulation of Phosphatidylinositol Phosphorylation in the Sarcoplasmic Reticular Ca ²⁺ -Transport ATPase by Vanadate M. VARSÁNYI, G. BEHLE, and M. SCHÄFER	310	New Pulvinic Acid Derivatives from <i>Pulveroboletus</i> Species (Boletales) (In German) R. MARUMOTO, C. KILPERT, and W. STEGLICH	363
Relative Hypertrehalosaemic Activities of Naturally Occurring Neuropeptides from the AKH/RPCH Family G. GÄDE	315	Volatiles from the Defensive Secretions of Two Rove Beetle Species (Coleoptera: Staphylinidae) K. DETTNER and G. SCHWINGER	366
Broadband, Non-Thermal Millimeter-Wave Influence on Giant Chromosomes CHR. KOSCHNITZKE, F. KREMER, L. SANTO, A. POGLITSCH, and L. GENZEL	321	Isolation of Beef Brain Phosphonolipids by Thin Layer Chromatography: Their Identification and Silicic Acid Column Chromatographic Separation M. C. MOSCHIDIS	369
The Anal Sac Secretion of Viverrids from the <i>Genus Genetta</i> J. JACOB and H. SCHLIEMANN	325	Contents of Number 4	
Some Remarks About Laser-Induced Mass Spectrometry of Bacteria J. ALBRECHT, E. W. SCHMID, and R. SÜSSMUTH	337	<i>Original Communications</i>	
Transepithelial Cytophagy by <i>Trichoplax adhaerens</i> F. E. Schulze (Placozoa) Feeding on Yeast H. WENDEROTH	343	Storage of Quinolizidine Alkaloids in Epidermal Tissues M. WINK	375
		Indole Alkaloids from <i>Ochrosia elliptica</i> Plant Cell Suspension Cultures K.-H. PAWELKA and J. STÖCKIGT	381
		Major Indole Alkaloids Produced in Cell Suspension Cultures of <i>Rhazya stricta</i> Decaisne K.-H. PAWELKA and J. STÖCKIGT	385

Determination of Hyoscyamine and Scopolamine in <i>Datura innoxia</i> Plants by High Performance Liquid Chromatography K.-H. PLANK and K. G. WAGNER	391	Characterization of a Defective Mutant of the Dahlemense Strain of Tobacco Mosaic Virus J. M. WERTZ, P. SMITAMANA, and S. SARKAR	477
Evidence for the Presence of Neutral Glycerylether Derivatives in Pollen Lipids of Pine Tree <i>Pinus halepensis</i> N. K. ANDRIKOPOULOS, A. SIAFAKA-KAPADAI, N. YANOVITS-ARGYRIADIS, and C. A. DEMOPOULOS	396	Electronmicroscopical Contrast by Palladium Chloride J. M. FERRER, A. TATO, and J. C. STOCKERT	483
A Chemical Investigation of <i>Pueraria mirifica</i> Roots J. L. INGHAM, S. TAHARA, and SR. Z. DZIEDZIC	403	Notes	
Characterization and Properties of Different Glucosyltransferases Isolated from Suspension-Cultured Cells of <i>Daucus carota</i> E. INGOLD and H. U. SEITZ	409	Cyanidin 3-Gentiobioside from Primary Leaves of Rye (<i>Secale cereale</i> L.) E. BUSCH, D. STRACK, and G. WEISSENBOCK	485
Conversion Rate of Ozone with Volatile Terpenes in the Surface Region of Conifer Needles (In German) H. RUSSI	421	¹ H-NMR Studies on the Effect of Spin Label on Lipids W. LOHMANN and B. KIEFER	487
Impact of UV-B Radiation on Photosynthetic Assimilation of ¹⁴ C-Bicarbonate and Inorganic ¹⁵ N-Compounds by Cyanobacteria G. DÖHLER, I. BIERMANN, and J. ZINK	426	The Macromolecular Structure of Collagen in Tendon Fibres of Dermatosparactic Animals E. MOSLER, W. FOLKHARD, W. GEERCKEN, O. HELLE, E. KNÖRZER, M. H. J. KOCH, CH. M. LAPIÈRE, H. NEMETSCHKE-GANSLER, B. NUSGENS, and TH. NEMETSCHKE	489
Pentachlorophenol Inhibits Photosynthetic Electron Flow and Quenches Chlorophyll Fluorescence after Preillumination CH. NIEHRS and J. AHLERS	433	Quasisynergism as Evolutionary Advance to Increase Repellency of Beetle Defensive Secretions K. DETTNER and R. GRÜMMER	493
The Diazo Reaction of Bilirubins and Phycorubins: A Quantitative Study W. KUFER, O. SCHMID, G. SCHMIDT, and H. SCHEER	437	Contents of Number 5/6	
Orientation Measurements on Ordered Multibilayers of Phospholipids and Sphingolipids from Synthetic and Natural Origin by ATR Fourier Transform Infrared Spectroscopy K. BRANDENBURG and U. SEYDEL	453	Original Communications	
The Dynamics of Bone Mineral in Some Vertebrates. F. C. M. DRIESSENS and R. M. H. VERBEECK	468	Pyoverdine Type Siderophores from <i>Pseudomonas aeruginosa</i> (In German) G. BRISKOT, K. TARAZ, and H. BUDZIKIEWICZ	497
A Serum-Free <i>in vitro</i> Culture System for Crayfish Organs G. GELLISSEN, M. TRAUB, and K.-D. SPINDLER	472	A New Biflavone and Further Flavonoids from the Moss <i>Hylocomium splendens</i> R. BECKER, R. MUES, H. D. ZINSMEISTER, F. HERZOG, and H. GEIGER	507
		4-O-β-D-Glucosides of Hydroxybenzoic and Hydroxycinnamic Acids – Their Synthesis and Determination in Berry Fruit and Vegetable B. SCHUSTER, M. WINTER, and K. HERRMANN	511
		Chemistry and Morphology of Epicuticular Waxes from Leaves of Five <i>Euphorbia</i> Species H. HEMMERS, P.-G. GÜLZ, and K. HANGST	521

¹ N-Acetyl-3-indolylmethylglucosinolate in Seedlings of <i>Tovaria pendula</i> Ruiz et Pav. H. SCHRAUDOLF and R. BÄUERLE	526	Effects of Adenosine-3':5'-monophosphate (cAMP) on the Activity of Soluble Protein Kinases in Maize (<i>Zea mays</i>) Coleoptile Homogenates B. JANISTYN	579
Changes in Fructose-2,6-bisphosphate Level during the Growth of Suspension Cultured Cells of <i>Catharanthus roseus</i> H. ASHIHARA	529	Effects of Pyridazinone Herbicides during Chloroplast Development in Detached Barley Leaves. III. Effects of SAN 6706 on Photosynthetic Activity and Chlorophyll-Protein Complexes G. LASKAY, E. LEHOCZKI, A. L. DOBI, and L. SZALAY	585
Biosynthesis of Pyoluteorin: A Mixed Polyketide-Tricarboxylic Acid Cycle Origin Demonstrated by [1,2- ¹³ C ₂]Acetate Incorporation D. A. CUPPELS, C. R. HOWELL, R. D. STIPANOVIC, A. STOESSL, and J. B. STOTHERS	532	Photocontrol of Chloroplast Lipids in Fern Gametophytes ST. KRAISS and A. R. GEMMRICH	591
The Expression of the Isoviteixin 7-O-Xylosylating Gene <i>gX</i> in <i>Silene pratensis</i> and <i>S. dioica</i> is Restricted to the Petals J. STEYNS and J. V. BREDERODE	537	Changes in the Stoichiometry of Photosystem II Components as an Adaptive Response to High-Light and Low-Light Conditions during Growth A. WILD, M. HÖPFNER, W. RÜHLE, and M. RICHTER	597
Ergosterol as a Biochemical Indicator of Fungal Infection in Spruce and Fir Needles from Different Sources W. F. OSSWALD, W. HÖLL, and E. F. ELSTNER	542	The Activation of the Cytochrome P-450 Dependent Monooxygenase System by Light D. MÜLLER-ENOCH and H. GRULER	604
Plant Defense Substances XXIX. Isolation, Characterization and Synthesis of Turgorines from <i>Gledisia triacanthos</i> L. (In German) H. SCHILDKNECHT, R. MULEY, G. M. KRESBACH, P. KUNZELMANN, and D. KRAUSS	547	Specificity of Rabbit Antibodies Elicited by Related Synthetic Peptides A. CHERSI, R. A. HOUGHTEN, F. CHILLEMI, R. ZITO, and D. CENTIS	613
Preparation of Pheromones by Simple Procedures H. K. MANGOLD and H. BECKER	555	Structure Investigations of Agonists of the Natural Neurotransmitter Acetylcholine, IV. X-Ray Structure Analyses of Trimethylpentylammonium-chloride and (4-Acetoxybutyl)trimethylammonium-iodide A. GIEREN and M. KOKKINIDIS	618
Increased Lipxygenase Activity is Involved in the Hypersensitive Response of Wheat Leaf Cells Infected with Avirulent Rust Fungi or Treated with Fungal Elicitor C. A. OCAMPO, B. MOERSCHBACHER, and H. J. GRAMBOW	559	Structure Investigations of Agonists of the Natural Neurotransmitter Acetylcholine, V. Structure-Activity Correlations for Cholinergic Stimulants Derived from Crystal Structures of Their Halides A. GIEREN and M. KOKKINIDIS	627
Stimulation of Photorespiration by the Carbonic Anhydrase Inhibitor Ethoxyzolamide in <i>Chlorella vulgaris</i> Y. SHIRAIWA and G. H. SCHMID	564	Structure Investigations of Agonists of the Natural Neurotransmitter Acetylcholine, VI. X-Ray Structure Analysis of Trimethyl[2-(propionyloxy)-ethyl]ammonium-iodide (O-Propionylcholine-iodide) A. GIEREN and M. KOKKINIDIS	641
The Photosynthetic Apparatus of <i>Ectothiorhodospira halochloris</i> . 2. Accessibility of the Membrane Polypeptides to Partial Proteolysis and Antenna Polypeptide Assignments to Specific Chromophores R. STEINER, A. ANGERHOFER, and H. SCHEER	571	Is the Calcium Pump Involved in Calcium Release? M. UNGEHEUER, A. MIGALA, and W. HASSELBACH	647

Selective Abolition of Sarcoplasmic Reticulum Vesicles' Calcium Releasing Mechanisms W. HASSELBACH, M. UNGEHEUER, A. MIGALA, and K. RITTER	652	of Pyridine-2,6-di(monothiocarboxylic Acid) from Pyridine-2,6-dicarboxylic Acid (In German) U. HILDEBRAND, K. TARAZ, and H. BUDZIKIEWICZ	691
The Sensitivity of the Ventral Nerve Photoreceptor of <i>Limulus</i> Recovers after Light Adaptation in Two Phases of Dark Adaptation I. CLASSEN-LINKE and H. STIEVE	657	A Furanoheliangolide in <i>Helianthus debilis</i> ; Implications for a Chemotaxonomy of the Genus <i>Helianthus</i> O. SPRING, V. KLEMT, K. ALBERT, and A. HAGER	695
<i>Notes</i>			
Antipeptide Antibodies: Do They Distinguish HLA-Alloantigens? A. CHERSI, R. A. HOUGHTEN, D. ZELASCHI, and C. CENCIARELLI	668	UDP-Glucose: Anthocyanidin/Flavonol 3-O-Glucosyltransferase in Enzyme Preparation from Flower Extracts of Genetically Defined Lines of <i>Matthiola incana</i> R. Br. M. TEUSCH, G. FORKMANN, and W. SEYFFERT	699
Erratum to A. PFITZNER, L. POLZ, and J. STÖCKIGT, Z. Naturforsch. 41c , 103–114 (1986)	671	Cyanidin 3-Oxalylglucoside in Orchids D. STRACK, E. BUSCH, V. WRAY, L. GROTHJAHN, and E. KLEIN	707
Contents of Number 7/8			
<i>Original Communications</i>			
Esters of Benzyl Alcohol and 2-Phenyl-ethanol-1 in Epicuticular Waxes from <i>Jojoba</i> Leaves P.-G. GÜLZ and F.-J. MARNER	673	Characterization of Glutamine Synthetase of Roots, Etiolated Cotyledons and Green Leaves from <i>Sinapis alba</i> (L.) R. MANDERSCHIED and A. WILD	712
Colletruncoic Acid Methyl Ester, a Unique Meroterpenoid from <i>Colletotrichum truncatum</i> A. STOESSL and J. B. STOTHERS	677	Are Polyphosphoinositides Involved in Signal Transduction of Elicitor-Induced Phytoalexin Synthesis in Cultured Plant Cells? H. STRASSER, CH. HOFFMANN, H. GRISEBACH, and U. MATERN	717
Complex Flavonoids from <i>Pityrogramma</i> Frond Exudates: Synthesis of Two Flavones with C–C-Linked Dihydrocinnamoyl Substituents M. IINUMA, K. HAMADA, M. MIZUNO, F. ASAI, and E. WOLLENWEBER	681	Herbal Insecticides III. Pyrethrin I in the Essential Oil of <i>Chrysanthemum balsamita</i> L. (In German) H. J. BESTMANN, B. CLASSEN, U. KOBOLD, O. VOSTROWSKY, and F. KLINGAUF	725
Occurrence of 2-(2-Hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3-one)- β -D-glucopyranoside in <i>Triticum aestivum</i> Leaves and Its Conversion into 6-Methoxy-benzoxazolinone H. J. GRAMBOW, J. LÜCKGE, A. KLAUSENER, and E. MÜLLER	684	Interference of Dimethazone with Formation of Terpenoid Compounds G. SANDMANN and P. BÖGER	729
6-(Hydroxythio)carbonylpyridine-2-carboxylic Acid and Pyridine-2-carboxylic Acid-6-monothiocarboxylic Acid as Intermediates in the Biosynthesis		Species-Specific Differences in Acetyl Coenzyme A Synthesis of Chloroplasts H.-J. TREEDE, B. RIENS, and K.-P. HEISE	733
		Nitrogen and Sulfur Starvation of the Cyanobacterium <i>Synechococcus</i> 6301. An Ultrastructural, Morphometrical, and Biochemical Comparison G. WANNER, G. HENKELMANN, A. SCHMIDT, and H.-P. KÖST	741

Conjugated Enamino Compounds, a New Molecular Probe for the Mechanism of Photosynthetic Electron Transport

T. ASAMI, N. TAKAHASHI, and S. YOSHIDA 751

Acyclo Nucleosides and Nucleotides: Synthesis, Conformation and Other Properties, and Behaviour in Some Enzyme Systems, of 2',3'-Seco Purine Nucleosides, Nucleotides and 3':5'-Cyclic Phosphates, Analogues of cAMP and cGMP

R. STOLARSKI, Z. KAZIMIERCZUK, P. LASSOTA, and D. SHUGAR 758

Azadirachtin, a Chemical Probe for the Study of Moulting Processes in *Rhodnius prolixus*

E. S. GARCIA, M. UHL, and H. REMBOLD 771

Comparative Enzymatic Degradation of H1 Subfractions from Syrian Hamster Tissues

E. HRABEC, A. PLUCIENNICZAK, and H. PANUSZ 776

Polymerization of Actin in the Absence and Presence of Cytochalasin B: Problems of Determining "Critical Concentration"

B. FUSSMANN and P. DANCKER 781

Proliferation Kinetics and Metabolic Features of *in vitro* Grown Ehrlich Ascites Tumor Cells in the Presence of Exogenous Pyruvate

W. KROLL, ST. POSTIUS, and F. SCHNEIDER 787

Triplet-Selective Chemistry: a Possible Cause of Biological Microwave Sensitivity

F. KEILMANN 795

Notes

Interaction between Spin Labels and DPPC Vesicles

W. LOHMANN, B. KIEFER, and W. SCHMEHL 799

Aspects of Mycobacterial Response to Beryllate Ions *in vitro*

H. J. MACCORDICK 802

Effect of Tuftsin on the Phagocytotic Activity of the Unicellular *Tetrahymena*. Does Primary Interaction Develop Imprinting?

G. CSABA, V. LÁSZLÓ, and P. KOVÁCS 805

Contents of Number 9/10

Original Communications

2-Hydroxy-4-methoxy-5-methyl Pyridine N-Oxide, an Al³⁺ Complexing Metabolite from *Pseudomonas cepacia* (In German)

ST. WINKLER, W. OCKELS, H. BUDZIKIEWICZ, H. KORTH, and G. PULVERER 807

Characterization of Volatile Constituents from Heterotrophic Cell Suspension Cultures of *Ruta graveolens*

M. JORDAN, C. H. ROLFS, W. BARZ, R. G. BERGER, H. KOLLMANNSSBERGER, and F. DRAWERT 809

Identification and Biosynthesis of Glucosylated and Sulfated Flavonols in *Flaveria bidentis*

L. VARIN, D. BARRON, and R. IBRAHIM 813

Substrate-Dependent Arylsulfatase Activity in the Cyanobacterium *Plectonema* 73110

S. MÜLLER and A. SCHMIDT 820

Volatile Fragrance Compounds from the Fungus *Gloeophyllum odoratum* (Basidiomycotina)

H.-P. HANSSEN, V. SINNEWELL, and W.-R. ABRAHAM 825

An Elicitor of the Hypersensitive Lignification Response in Wheat Leaves Isolated from the Rust Fungus *Puccinia graminis* f. sp. *tritici*. I. Partial Purification and Characterization

B. MOERSCHBACHER, K. H. KOHEL, U. NOLL, and H. J. REISENER 830

An Elicitor of the Hypersensitive Lignification Response in Wheat Leaves Isolated from the Rust Fungus *Puccinia graminis* f. sp. *tritici*. II. Induction of Enzymes Correlated with the Biosynthesis of Lignin

B. MOERSCHBACHER, B. HECK, K. H. KOHEL, O. OBST, and H. J. REISENER 839

Metabolic Conversions of Trichothecene Mycotoxins: Biotransformation of 3-Acetyldeoxynivalenol into Fusarenon-X

N. C. P. BALDWIN, B. W. BYCROFT, P. M. DEWICK, and J. GILBERT 845

Biochemical Properties and Crystal Structure of Ethylmethylglyoxal Bis(guanyldihydrazone) Sulfate — an Extremely Powerful Novel Inhibitor of Adenosylmethionine Decarboxylase H. ELO, I. MUTIKAINEN, L. ALHONEN-HONGISTO, R. LAINE, J. JÄNNE, and P. LUMME	851	(R)Mandelonitrile and Prunasin, the Sources of Hydrogen Cyanide in All Stages of <i>Paropsis atomaria</i> (Coleoptera: Chrysomelidae) A. NAHRSTEDT and R. H. DAVIS	928
Protein Phosphorylation — Dephosphorylation in the Cytosol of Pea Mesophyll Cells R. HRACKY and J. SOLL	856	Latitude Dependent Circadian Rhythms of Carabid Beetles G. LEYK and W. MARTIN	935
Diethyldithiocarbamate, a New Photosystem I Electron Donor of Mehler-Type Hill Reactions B. L. UPHAM and K. K. HATZIOS	861	Notes	
Photodestruction of Endogenous Porphyrins in Relation to Cellular Inactivation of <i>Propionibacterium acnes</i> T. B. MELØ and G. REISÆTER	867	New Flavonoids from the Exudate of <i>Baccharis bigelovii</i> (Asteraceae) F. J. ARRIAGA-GINER, E. WOLLENWEBER, and D. HRADETZKY	946
The Photosynthetic Apparatus of <i>Ectothiorhodospira halochloris</i> . 3. Effect of Proteolytic Digestion on the Photoactivity R. STEINER, B. KALUMENOS, and H. SCHEER	873	The C-Glycosylflavone Pattern of <i>Passiflora incarnata</i> L. H. GEIGER and K. R. MARKHAM	949
Phenolic Herbicides: Correlation between Lipophilicity and Increased Inhibitory Sensitivity of Thylakoids from Higher Plant Mutants J. DURNER, A. THIEL, and P. BÖGER	881	Antiproliferative Activity of Derivatives of <i>trans</i> -Bis(salicylaldoximate)copper(II) <i>in vitro</i> . Some <i>in vivo</i> Properties of the Parent Compound H. ELO and P. LUMME	951
Biosynthesis and Turnover of Cell Wall Glycoproteins during the Vegetative Cell Cycle of <i>Chlamydomonas reinhardtii</i> J. VOIGT	885	Effect of Tumour Regression on Serum and Tissue Copper Concentration in Mice Bearing Induced Fibrosarcoma P. K. CHAKRAVARTY, A. GHOSH, and J. R. CHOWDHURY	956
Nucleic Acid-Binding Activities of the Intermediate Filament Subunit Proteins Desmin and Glial Fibrillary Acidic Protein C. E. VORGAS and P. TRAUB	897	Contents of Number 11/12	
Soluble and Insoluble Rat Liver Chromatin is Different in Structure and Protein Composition R. BRUST	910	Contents of Nos 1–12	III–XII
Specific Binding of Calcium to Soluble Chromatin R. BRUST	917	Original Communications	
On the Direct Observation of Water-Fluxes in Tissues and Leaves U.-A. HIRTH and R. LAWACZEK	923	Volatiles from Liquid Cultures of <i>Lentinellus cochleatus</i> (Basidiomycotina) H.-P. HANSSEN and W.-R. ABRAHAM	959
		Biosynthesis of Flavor Compounds by Microorganisms. 6. Odorous Constituents of <i>Polyporus durus</i> (Basidiomycetes) R. G. BERGER, K. NEUHÄUSER, and F. DRAWERT	963
		<i>Rhizomnium magnifolium</i> and <i>R. pseudopunctatum</i> , the First Mosses to Yield Flavone Glucuronides R. MUES, G. LEIDINGER, V. LAUCK, H. D. ZINSMEISTER, T. KOPONEN, and K. R. MARKHAM	971

Flavonoic Constituents of <i>Rhamnus lycioides</i> L. M. PAYÁ, S. MÁÑEZ, and A. VILLAR	976	Adsorbent Culture of Tobacco Cell Suspensions with Different Adsorbents R. MAISCH, B. KNOOP, and R. BEIDERBECK	1040
Green Algae (<i>Scenedesmus obliquus</i>) Contain Three Thioredoxins of Regular Size P. LANGLOTZ, W. WAGNER, and H. FOLLMANN	979	Changes in Levels of Cellular Constituents in Sus- pension Culture of <i>Catharanthus roseus</i> Associ- ated with Inorganic Phosphate Depletion T. UKAJI and H. ASHIHARA	1045
A Simple and Rapid Method for Isolation of 124 kDa Oat Phytochrome R. GRIMM and W. RÜDIGER	988	Flow Cytometric DNA-Analysis of Plant Protoplasts Stained with DAPI I. ULRICH and W. ULRICH	1052
Investigation of the Peptide Chain of 124 kDa Phyto- chrome: Localization of Proteolytic Fragments and Epitopes for Monoclonal Antibodies R. GRIMM, F. LOTTSPEICH, H. A. W. SCHNEIDER, and W. RÜDIGER	993	Evidence for the Intercalation of Thalidomide into DNA: Clue to the Molecular Mechanism of Tha- lidomide Teratogenicity? H. P. KOCH and M. J. CZEJKA	1057
Isolation and Characterization of 3 Protochlorophyl- lides from Pigment Mutant C-2A' of <i>Scenedesmus obliquus</i> K. KOTZABASIS and H. SENGER	1001	Formation and Structure of Radicals from D-Ribose and 2-Deoxy-D-ribose by Reactions with $\text{SO}_4^{\cdot-}$ Rad- icals in Aqueous Solution. An <i>in-situ</i> Electron Spin Resonance Study J. N. HERAK and G. BEHRENS	1062
Phosphoenolpyruvate Carboxylase from Maize Leaves. Studies Using β -Methylated Phospho- enolpyruvate Analogues as Inhibitors and Sub- strates D. H. GONZÁLEZ and C. S. ANDREO	1004	Alkylene-bis-isothiocyanates: Novel Insect Growth Regulators G. MATOLCSY, I. UJVÁRY, L. M. RIDDIFORD, and K. HIRUMA	1069
Purification of the Chloroplast Pyruvate Dehydro- genase Complex from Spinach and Maize Meso- phyll H.-J. FREEDE and K.-P. HEISE	1011	C_9 Aliphatic Aldehydes: Possible Sex Pheromone from Male Tropical West African Shield Bug, <i>Sphaerocoris annulus</i> A. J. E. GOUGH, D. E. GAMES, B. W. STADDON, D. W. KNIGHT, and T. O. OLAGBEMIRO	1073
On the Role of Magnesium in the Reaction of the Pyruvate Kinase from <i>Salmonella typhimurium</i> C. GARCIA-OLALLA and A. GARRIDO-PERTIERRA	1018	Individual Variation in the Sex Pheromone Compo- nents of the False Codling Moth, <i>Cryptophlebia leucotreta</i> (Lepidoptera: Tortricidae) A. B. ATTYGALLE, J. SCHWARZ, O. VOSTROWSKY, and H. J. BESTMANN	1077
Content and Metabolism of Indole-3-acetic Acid (IAA) in Healthy and Rust-Infected Wheat Leaf Segments G. WIESE and H. J. GRAMBOW	1023	Screening and Use of Sex Attractants in Monitoring of Geometrid Moths in Bulgaria M. A. SUBCHEV, J. A. GANEV, O. VOSTROWSKY, and H. J. BESTMANN	1082
The Production of Pyrenocines A and B by a Novel <i>Alternaria</i> species B. TAL and D. J. ROBESON	1032	Relative Potencies of Antagonists of the Luteinizing Hormone Releasing Hormone with Lys^8 and Arg^8 and Substitutions in Positions 3, 5, 6, 7 and 8 K. FOLKERS, C. BOWERS, P.-F. L. TANG, M. KOB- OTA, X. SHAO-BO, W. BENDER, and L. YIN-ZENG	1087
Sensitivity of a Phototrophic Bacterium to the Herbi- cide Sulfometuron Methyl, an Inhibitor of Branched Chain Amino Acid Biosynthesis I. SCHNEIDER and J.-H. KLEMME	1037		

Effect of External Calcium Concentration on the Intensity Dependence of Light-Induced Membrane Current and Voltage Signals in Two Defined States of Adaptation in the Photo-Receptor of <i>Limulus</i> H. STIEVE, H. GAUBE, and J. KLOMFASS	1092	An Improved Procedure for the Quantitative Estimation of the Rust Fungus in Infected Plant Tissue G. WIESE, D. HUGO-WISSEMAN, and H. J. GRAMBOW	1127
Effects of Low Frequency Magnetic Fields on Chick Embryos. Dependence on Incubation Temperature and Storage of the Eggs J. P. JUUTILAINEN	1111	Low Molecular Mass Inhibitors from Calf Thymus Selective for T-Lymphocyte Proliferation H. P. MATTHIESSEN and H. R. MAURER	1131
<i>Notes</i>		The Effect of Volatile Anesthetics on Giant Neurons in the Lobula Plate in the Fly K. KIRSCHFELD	1137
Preliminary Studies towards a Monograph of the Lichen Family Roccellaceae Chev. VII. Secondary Products and Relationships of the Genera <i>Combea</i> de Not. and <i>Schizopelte</i> T. M. Fries (In German) G. FOLLMANN and M. GEYER	1117	Microfilament-Supported Macrovilli in the Hindgut of the Polychaete <i>Dinophilus gyrotilatus</i> U. OSTER	1139
Rearrangement of Glaucolide A into Vernojalcanolide 8-O-Methacrylate M. MARTÍNEZ, A. SÁNCHEZ, G. LÓPEZ, and P. JOSEPH-NATHAN	1119	Comment on: Is there an Equilibrium between Ascorbic and Dehydroascorbic Acids? H. W. MUELLER	1145
Thin Layer Chromatographic and IR Spectral Evidence for the Presence of Phosphonolipids in Human Sperm M. C. MOSCHIDIS	1121	<i>Report</i>	
Evidence for the Presence of Glycerophosphonolipids in the Land Snail <i>Eobania vermiculata</i> M. C. MOSCHIDIS	1124	The Glio-Axonal Interaction and the Problem of Regeneration of Axons in the Central Nervous System – Concept and Perspectives H. WOLBURG, J. NEUHAUS, and A. MACK	1147
		Subject Index	1157
		Authors Index	1181

The Photosynthetic Apparatus of *Ectothiorhodospira halochloris*

2. Accessibility of the Membrane Polypeptides to Partial Proteolysis and Antenna Polypeptide Assignments to Specific Chromophores

R. Steiner*, A. Angerhofer⁺, and H. Scheer*

* Botanisches Institut der Universität München, Menzinger Straße 67, D-8000 München 19

⁺ Physikalisches Institut, Teilinstitut 3, Universität Stuttgart, Pfaffenwaldring 57, D-7000 Stuttgart 80

Z. Naturforsch. **41c**, 571–578 (1986); received January 9, 1986

Bacterial Photosynthesis, *Ectothiorhodospira*, Membrane Topology, Antenna Polypeptides, Fluorescence, Circular Dichroism, Energy Transfer

E. halochloris thylakoids and spheroplasts were treated with trypsin, thermolysin or proteinase K to determine which proteins are exposed at the different membrane surfaces. Based on SDS polyacrylamide analysis, all 9 polypeptides are exposed on the cytoplasmic side. Only one (28 kDa) is accessible from the periplasmic side. This polypeptide is generally isolated as the H-subunit of the reaction centers of photosynthetic bacteria, but is in the case of *E. halochloris* rather isolated with the antenna (B 800/1020) (Steiner and Scheer, Biochim. Biophys. Acta **807**, 278, 1983).

Proteolysis is accompanied by a shift of the absorption band at longest wavelengths from 1020 to 960 nm (B 800/960), which upon standing is shifted further to 680 nm ("B" 800/680). The spectral changes are similar to the ones reported earlier for treatment with acid, and are also inducible with urea. The correlation of SDS-PAGE and absorption spectroscopy shows, that the chromophores absorbing at 1020 nm are transformed simultaneously with the degradation of the 6.5 kDa (= α) polypeptide.

Introduction

Much progress has been made in recent years in understanding the topology of photosynthetic membranes in purple bacteria. The techniques used included biochemical methods of different specificities and advantages like labelling with antibodies [1], radioiodination [2], photoaffinity labelling [3], cross-linking experiments [4–6] or proteolytic digestion [7–9]. They also included diffraction methods, like high-resolution electron microscopy [10–12] and most recently x-ray diffraction of isolated reaction centers [13].

Most of this work was focused on only a small number of closely related bacterial species. Particular emphasis has been placed on the Rhodospirillales [14] e.g. *Rs. rubrum*, *Rp. spheroides*, *Rp. capsulata* and *Rp. viridis*. Much less is known about species

from other genera [15], and their structural relations to the Rhodospirillales. We have recently begun to study the photosynthetic apparatus of *E. halochloris* [16], an alcalophilic and extremely halophilic bacteriochlorophyll *b* containing organism.

The main difference as compared to e.g. *Rp. viridis* is a second near-infrared absorption band besides the common one at 1020 nm, peaking at 800 nm with a shoulder at 830 nm. In a previous study [17], it was shown that the native ("high-pH") form ($\lambda_{\max} = 1020$ and 800 nm) is reversibly transformed below pH 6.5 to a form absorbing at 960 and 800 nm ("low-pH" form). The 800/830 nm band remained unchanged by this treatment [17]. The pigments relating to the 960 nm band in the "low-pH" form are not very stable, and are oxidized irreversibly upon addition of more acid or even incubating at ambient temperature. The newly formed absorption peaking at about 680 nm is typical for the chlorophyll *a*-related oxidation products of bchl *b* [18]. Similar, albeit irreversible spectral changes have now been observed upon (partial) proteolysis of *E. halochloris* membranes. Here we wish to report results pertaining to the orientation and exposition of the photosynthetic membrane proteins of this organism, and combine data from SDS-gel electrophoresis and spectroscopy,

Abbreviations: Rp, *Rhodopseudomonas*; Rs, *Rhodospirillum*; E, *Ectothiorhodospira*; bchl, bacteriochlorophyll; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonylfluoride; EDTA, ethylenediaminetetraacetate; cd, circular dichroism; LHP, light-harvesting-protein; RC, reaction center.

Reprint requests to Prof. Dr. H. Scheer.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0500–0571 \$ 01.30/0

in order to relate spectrally distinct chromophores to certain polypeptides.

Material and Methods

E. halochloris was grown anaerobically in the medium of Imhoff and Trüper, with the differences described earlier [17]. The cells were harvested by centrifugation ($14000 \times g$) and washed once with Tris-buffer (10 mM, pH = 7.5). Thylakoids in which mainly the cytoplasmic surface is exposed, were prepared by the method of Feher and Okamura [19] and checked by light microscopy for homogeneity. Spheroplasts (rightside-out particles) were prepared after a modified method of Michels and Konings [20]: Harvested and washed cells were homogenized in a glass potter in Tris-buffer. The crude extract was treated with lysozyme (0.5 mg/ml) and EDTA (final concentration 5.5 mM) and stirred at room temperature for about 3 hours [21]. It is necessary to check the formation of the particles by microscopy (characteristic swelling; addition of more lysozyme if necessary). The suspension was then sonicated twice for one minute, centrifuged twice like chromatophores and adjusted to an absorption of 50 (1020 nm; 1 cm cuvettes).

For controlled proteolytic digestion, 500 μ l of the membrane particles (chromatophores or spheroplasts) were incubated with increasing amounts of different proteases: proteinase K, trypsin or thermolysin. The digestion was stopped with PMSF (2 ml saturated solution in acetone/0.5 ml incubation mixture), trypsin-inhibitor (400 mg/ml incubation buffer, added as a solid) or EDTA (1.0 M final concentration), respectively. The samples were then centrifuged ($14000 \times g$) washed three times with Tris-buffer and analyzed.

SDS-gel electrophoresis was done on polyacrylamide gels (PAGE) with a linear gradient (11.5–16.5% acrylamide), modified from Laemmli [22], as described earlier [17]. For calibration a standard set of hydrophilic proteins was used (bovine serum albumin; hen egg albumin; lactoglobulin; pepsin; trypsinogen and lysozyme) and in addition hydrophobic peptides (*Rp. spheroides* B 800/850 antenna) of known molecular weights [23]. Gels were scanned after staining with Coomassie brilliant blue G on a scanner TCD (Vitatron).

Absorption spectra were measured on a DMR 22 (Zeiss, Oberkochen) or a ZWS II spectrophotometer

(Sigma, Berlin) connected to a BS 8000 intelligent recorder (Bryans, Mitcham). CD-spectra were obtained on a dichograph V (ISA, Unterhaching) equipped with a silex data handling system (Leanord, Lille) with a modified software. Fluorescence-emission spectra were obtained on a home-built fluorimeter equipped with a liquid-helium cryostat as described elsewhere [24] and are uncorrected.

All chemicals were reagent grade. Trypsin and proteinase K were purchased from Merck, Darmstadt, lysozyme and trypsin inhibitor from Serva, Heidelberg, thermolysin from Boehringer, Mannheim, and the SDS-PAGE calibration set from Sigma, München.

Results

Absorption spectra

E. halochloris has two major near infrared absorptions at 1020 and 800 nm. When thylakoids are treated with proteases, the 1020 nm absorption is gradually transformed into a 960 nm absorption, whereas the 800/830 nm band remains unchanged (Fig. 1). Qualitatively these changes are identical irrespective of the type of protease used (trypsin, proteinase K or

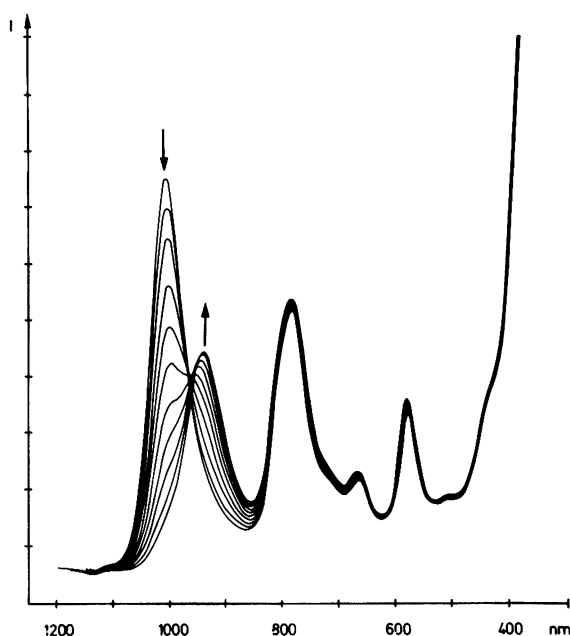


Fig. 1. Titration of *E. halochloris* thylakoids with increasing amounts of trypsin in 10 mM Tris buffer, pH = 8.0. The final concentration of trypsin was 100 μ g/2 ml thylakoid suspension with $A_{1020} = 0.7/\text{cm}$.

thermolysin). The absorption changes are similar to the ones observed earlier upon lowering the pH [17], with two differences: Firstly the reaction is irreversible and thus due to a true proteolysis and not a pH-change induced by any action of the enzymes. Secondly the effectiveness of proteolysis is strongly dependent on the membrane orientation. In contrast to the acid induced absorption change, proteolysis works with thylakoids only, but not with spheroplasts. Similar to the pH-changes, the 960 nm form is again only metastable and transforms within a few minutes to yield the chlorophyll *a*-related oxidation-products of bchl *b* (Fig. 2) [18].

There is yet a third means of inducing the 1020 → 960 nm transformation in *E. halochloris*, e.g. by the treatment with urea. Globular, hydrophilic proteins are generally fully denatured (unfolded) by treatment with 8 M urea. The membrane proteins of *E. halochloris* are much more stable, if judged from their absorption spectra ≤ 900 nm. However, the 1020 nm band is again sensitive and transformed increasingly rapidly with increasing urea concentrations (4–8 M) to the 960 nm form. The absorption changes (Fig. 3) are identical to the ones induced by proteolysis or acid. Only thylakoids are susceptible to this treatment which is similar to the results ob-

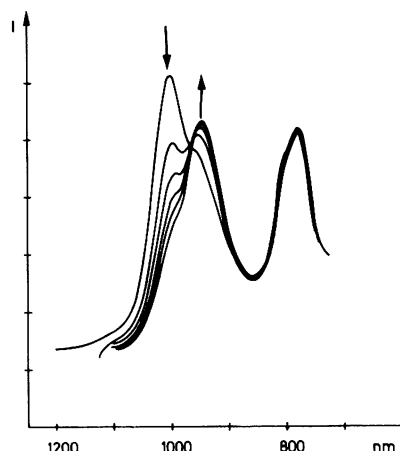


Fig. 3. Titration of *E. halochloris* thylakoids with increasing concentrations of urea (5.0, 5.5, ..., 7.5 M urea) in 10 mM Tris buffer, pH = 8.0.

tained upon proteolysis. We have also been unable to reverse the urea-induced transformation. Possibly the time necessary to remove the denaturant (e.g. 10 min necessary for by filtration over a short column of desalting gel) is already too long to prevent the further and principally irreversible oxidation of the bchl *b* absorbing at 960 nm.

Fluorescence spectra

In order to further compare the 960 nm forms obtained by the different treatments (acid-, urea- or protease), low-temperature fluorescence spectra were recorded:

The low temperature (5 K) emission spectra of all three forms show a single band peaking at 1007 nm (5 K), whereas the original emission of *E. halochloris* is at 1066 nm (Fig. 4). If the bacteriochlorophyll *b* chromophores absorbing at longest wavelengths are the emitters, this corresponds to Stokes-shifts of about 46 nm, for both the original chromophores (λ_{max} , absorption = 1020 nm) and the modified ones (λ_{max} , absorption = 960 nm). The fluorescence excitation spectra below 920 nm are identical for all four samples with respect to band positions and intensities (Fig. 5). This is further support that the chromophores absorbing at 800/830 nm are not affected by any of these treatments. In all three forms, there is also an efficient energy transfer from the 800/830 nm absorbing chromophores to the 960 nm ones, if judged from the excitation peak around 800 nm for

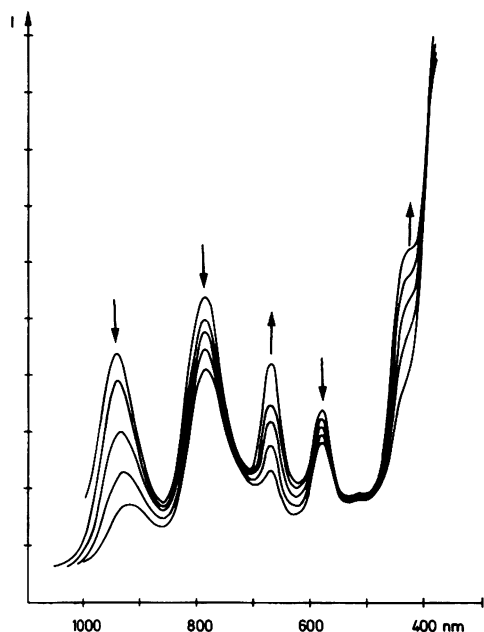


Fig. 2. Transformation of the B 800/960 form upon standing to the "B" 800/680 form. Sample as in Fig. 1. The time between each curve was 5 minutes.

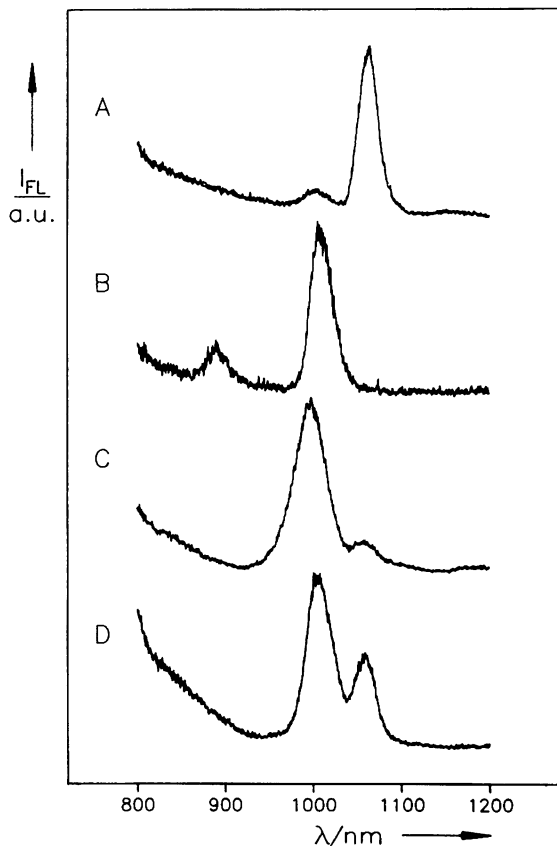


Fig. 4. Fluorescence emission spectra (excitation: 600 nm) of *E. halochloris* chromatophores at 5 K. A. Untreated chromatophores (λ_{max} , absorption = 1020 nm); B. acid induced "low-pH" form (λ_{max} , absorption = 960 nm); C. protease treated (λ_{max} , absorption = 960 nm); D. urea treated (λ_{max} , absorption = 960 nm).

the 1007 nm emission. In the visible and near-UV spectral range, the excitation spectra are indicative of (monomeric) bacteriochlorophyll *b* and the absence of bacteriopheophytin *b* (no bands around 530 nm).

Circular dichroism

The three species modified by either low pH, proteolysis or urea are also identical with respect to their circular dichroism spectra. All three 960 nm forms show an "S"-shaped band centered around 974 nm with extrema around 990 and 940 nm (data not shown). As discussed previously for the low-pH form [17], these spectra can be rationalized by (a minimum of) two strongly interacting bchl *b* molecules absorbing around 960 nm.

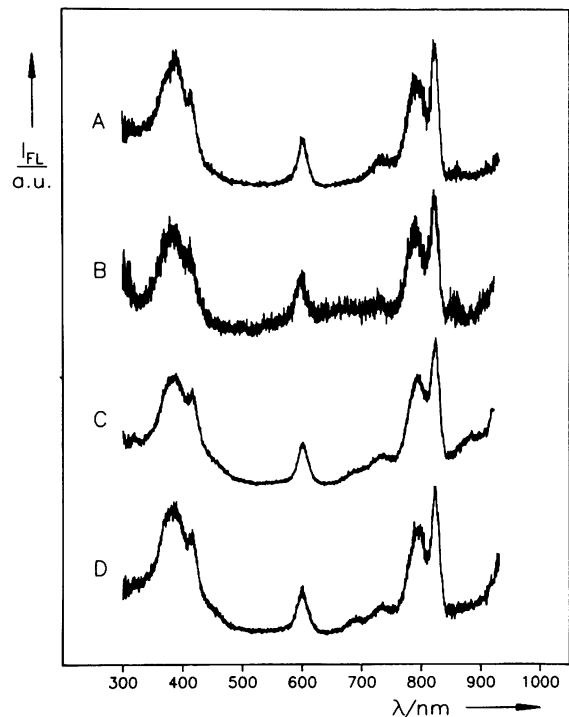


Fig. 5. Excitation spectra, corresponding to the emission spectra in Fig. 4. Emission at 1020 (A) and 960 nm (B, C, D), respectively.

The 800/830 nm region shows an M-shaped signal, which is related to (a minimum of) three chromophores. The magnitude of this band is the only indication, that at least some changes take place, too, in the pigments absorbing around 800 nm during the transformation of the B 800/1020 to the B 800/960 forms. The cd pattern of the latter in the 800 nm region is similar in shape, but reduced in intensity by about 50% as compared to the 800/1020 nm form.

SDS-gel electrophoresis

In order to relate distinct changes in the polypeptide pattern to the spectroscopic changes and hence to chromophores with distinct absorptions, the incubation with protease was followed in parallel by SDS-polyacrylamide gel electrophoresis and absorption spectroscopy. The SDS-PAGE of *E. halochloris* membranes shows four bands in the "high-molecular weight" range ≥ 16 kDa (34.0, 28.0, 23.8 and 16.8 kDa). They have similar relative, but generally higher mobilities than the polypeptides of the reaction center polypeptides from *Rp. viridis* (Table I)

and were therefore tentatively assigned to the RC subunits cytochrome *c*, H, M and L.

Table.

<i>Rp. viridis</i> (a)		<i>E. halochloris</i> (c)
38.0	cytochrome <i>c</i>	34.0
33.0	H	28.0
27.0	M	23.8
24.0	L	16.8
	?	15.2
	?	14.5
11.0 (6.848, b)	α -LHP	13.5 (6.5, d)
8.0 (6.138, b)	β -LHP	13.0 (6.0, d)
6.0 (4.001, b)	γ -LHP	12.2 (4.5, d)

(a) SDS-PAGE data from Jay *et al.*, 1983 [25].

(b) Data from primary structure analysis [26].

(c) Data from SDS-PAGE (this work), calibrated with hydrophilic globular proteins.

(d) Calibrated with B 800/850 subunits from *Rp. sphaeroides*.

In the low-molecular weight region (≤ 16 kDa) two barely resolved major bands appear in addition to a weakly staining third band (Figs. 6, 7). Since they are isolated with the antenna fraction, they have been assigned to the light-harvesting complex [17]. This composition is again similar to that of *Rp. viridis* (Table I). The only exception is that a 28.0 kDa band is isolated with the antenna from *E. halochloris*, whereas a polypeptide of this size is generally isolated as the "H"-subunit of the reaction center [17].

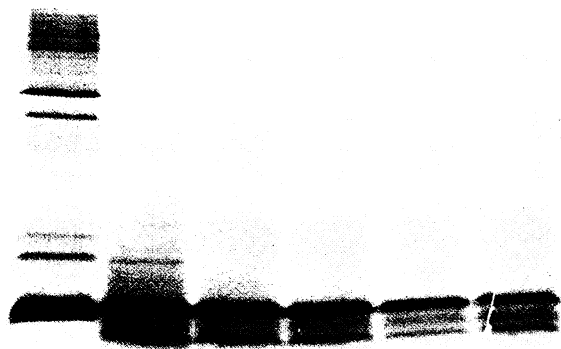


Fig. 6. SDS-PAGE of chromatophores from *E. halochloris*, incubated with proteinase K. 3 ml of the chromatophores ($E_{1020} = 50$) were incubated with 0.5 mg proteinase K for 0, 5, 15, 30, 45 and 75 from left to right minutes at room temperature. See Fig. 7 for assignment of the bands.

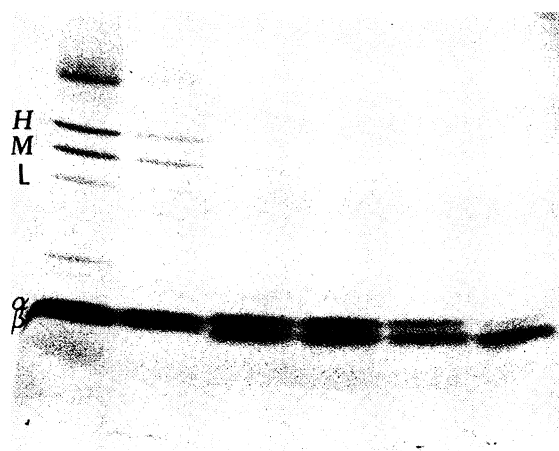


Fig. 7. SDS-PAGE of trypsin incubate chromatophores of *E. halochloris*, that shows the degradation of the 6.5 kDa polypeptide. 0.5 ml chromatophores ($E_{1020} = 50$) were incubated with 650, 700, 750, 800, 900, 1000 μ g trypsin from left to right for 30 minutes at room temperature. The assignments of the main bands is indicated on the left margin. See text for the molecular weights.

The apparent molecular weights of these membrane proteins are shown in Table I.

When thylakoids of *E. halochloris* are incubated with increasing amounts of proteases, the polypeptide bands were degraded in a specific sequence. SDS-PAGE gels of the digestion with trypsin are shown in Fig. 6, but similar results are obtained with proteinase K and thermolysin (not shown).

In the "high molecular weight" region the cytochrome band disappears first, followed rapidly by H, L, and much more slowly by M. This means that in the membrane the M subunit is the most stable peptide of the RC (within the limits of resolution of our gels, *viz.* ± 5 amino acid residues). Since the sequence of digestion is the same with all three proteases used, in a first approximation only the accessibility of the proteins is important rather than a distinct amino-acid sequence. It should be noted that in the *bchl a* containing organisms *Rp. capsulata* [27] and *Rs. rubrum* [28, 29] the L subunit of the RC is the most stable one. However, the assignment of RC bands by mobility alone is insufficient and further confirmation of this assignment is necessary.

The lower-molecular-weight polypeptides of the light-harvesting complex were digested much slower than the RC polypeptides. This different time course of the digestion has helped us to assign the fragments

to either the RC or LH polypeptides. A gel with high resolution in this region is shown in Fig. 6. The 6.0 kDa band is least stable. It is degraded only a little to yield a band with an apparent molecular weight of 5.3 kDa, while the intensity of the 6.5 kDa band remains constant. Secondly, the 6.5 kDa band is attacked. The fate of the fastest migrating γ -peptide (4.5 kDa) is difficult to assess quantitatively, because it stains only weakly with Coomassie blue. When incubating the isolated antenna complex of *E. halochloris* all bands disappear more or less simultaneously. This can be rationalized by the protection of the hydrophobic surfaces of the peptides in the chromatophore membrane which is lost in the solubilized complex.

Discussion

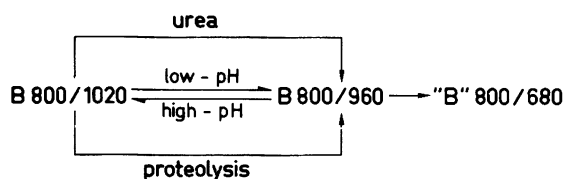
The spectroscopic results suggest, that the different treatments of *E. halochloris* membranes, e.g. lowering of the pH below 6.5 [17], proteolysis and incubation with urea, transform the pigment arrangement within the antenna apparatus in the same or at least in a very similar manner. Of these reactions, only the one induced by acid is reversible. A reversion may principally be possible, too, for the treatment with urea. However, the fastest time achieved for its removal (≈ 10 min) is comparable to the half-life of the 960 nm chromophores and turned out to be ineffective. These results are summarized in Scheme 1.

The proteolysis has been studied in more detail. Two distinct results regarding the antenna structure within the membrane of *E. halochloris* can be drawn. The first is a correlation of the chromophores absorbing at different wavelengths to distinct polypeptides. The absorption shift from 1020 to 960 nm occurs simultaneous to the digestion of the 6.5 kDa ($= \alpha$ -) subunit, and after the proteolysis of the 6.0 kDa antenna polypeptide and any of the reaction center polypeptides. The chromophores absorbing at 1020 nm in the native antenna are thus either bound

to the α -subunit or their spatial arrangement is at least strongly influenced by it. The role of the γ -subunit is difficult to assess because it stains only weakly and unreliably. Like in *Rp. viridis* [26] it does not carry a histidine residue (Brunisholz, unpublished). Since histidine is currently assumed to be the common binding amino acid for the chromophore in the bacterial antenna polypeptides [23, 30, 31], the γ -subunit is considered a structural polypeptide, to which the crystallinity of the membranes [10–12] in bacteriochlorophyll *b*-containing bacteria may be related [26]. The chromophores absorbing around 800 nm should then be bound to the other antenna polypeptides, most likely to the β -subunit (6.0 kDa). However, proteolytic cleavage of a small (≈ 0.5 kDa) oligopeptide from this subunit does not affect significantly the 800/830 nm absorption.

The relative intensities of the chromophores absorbing at 1020 to the ones absorbing at 800 nm is roughly 2:1 and thus similar to the relative intensities of the chromophores absorbing at 850 and 800 nm, respectively, in type I B 800/850 antenna complexes of bchl *a*-containing species, e.g. *Rp. spheroides* [32]. In both complexes, the chromophores absorbing at the longest wavelengths are bound to the heavy antenna polypeptide [23]. The most significant difference is the coupling of the chromophores inferred from the circular dichroism spectra. In the B 800/850 complex of *Rp. spheroides*, four excitation coupled bacteriochlorophyll *a* molecules were discussed as being responsible for the 850 nm absorption and orientated parallel to the α -helix of the polypeptides, and two weakly coupled chromophores orientated perpendicular for the 800 nm absorption [33]. In *E. halochloris*, a minimum of three strongly coupled chromophores is responsible for the 800 nm band and at least 2 for the 1020 nm absorption [17]. No data are available on their orientation with respect to the membrane.

The second aspect of the proteolytic digestion concerns the topology of the antenna of *E. halochloris*. Only the H-subunit of the reaction center is digested if spheroplasts are treated with proteases. In thylakoids having mainly the cytoplasmic side exposed, all polypeptides of the antenna (with the possible exception of the weakly staining γ -subunit) and all reaction center polypeptides are accessible to proteases. This would indicate, that only the H-subunit is spanning the photosynthetic membrane, as far as its accessibility to proteases is concerned. Similar



Scheme 1.

conclusions have been drawn from proteolytic studies with the bchl *a* containing *Rs. rubrum* [8, 9, 34, 35], *Rp. spheroides* [36] and *Rp. capsulata* [5, 27], as well as with the bchl *b*-containing *Rp. viridis* [34]. In all cases, the reaction center polypeptides are more labile than the ones related to the antennas, and only the H-subunit has always been found to be accessible from either side of the membrane. The validity of the latter results has been questioned, however, by the x-ray data of Deisenhofer *et al.* [13] on *Rp. viridis* reaction centers. The major part of the H-subunit is located on the cytoplasmic surface, with only a single α -helix spanning the membrane and less than 10 amino acid residues being exposed on the periplasmic side. From the x-ray data, it is rather the L- and M-subunits which are transmembrane polypeptides. These discrepancies may be due to (i) a true species difference, (ii) a misassignment of the H band from SDS-PAGE derived molecular weights, or (iii) a fortuitous overlap of a large H fragment with either the M or L band. The third possibility should be indicated by an increased intensity of

either the M or L band, which was not observed. The second possibility can presently not be decided upon for the lack of sufficient sequence data. Species differences are indicated by differential proteolytic sensitivities of polypeptides of the photosynthetic membranes of the species cited above. They are also supported by labeling methods exhibiting different selectivities or steric requirements, *e.g.* by iodination [2, 5, 25, 35] and by immunochemical data [5, 25, 39]. However, a comparison of the results obtained in different laboratories and with different biochemical techniques is difficult, and comparative work with different species under otherwise identical conditions is necessary. Such work with *E. halochloris* and the much better known *Rp. viridis* is in progress.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, Bonn (SFB 143). We thank H. C. Wolf (Stuttgart) and W. Rüdiger (München) for continuing support.

- [1] D. W. Reed, D. Raveed, and M. Reporter, *Biochim. Biophys. Acta* **387**, 368 (1975).
- [2] H. Zürrer, M. Snozzi, K. Hanselmann, and R. Bachofen, *Biochim. Biophys. Acta* **460**, 273 (1977).
- [3] E. Odermatt, M. Snozzi, and R. Bachofen, *Biochim. Biophys. Acta* **591**, 372 (1980).
- [4] J. Y. Takemoto, J. Peters, and G. Drews, *FEBS Lett.* **142**, 227 (1982).
- [5] J. Peters and G. Drews, *Eur. J. Cell Biol.* **29**, 115 (1983).
- [6] J. Peters, J. Y. Takemoto, and G. Drews, *Biochem.* **22**, 5660 (1983).
- [7] V. Wiemken and R. Bachofen, *Biochim. Biophys. Acta* **681**, 72 (1982).
- [8] V. Wiemken, R. Brunisholz, H. Zuber, and R. Bachofen, *FEMS Microbiol. Lett.* **16**, 297 (1983).
- [9] K. Tsuji, K. Tanaka, K. Sakata-Sogawa, G. Soe, T. Kakuno, J. Yamashita, and T. Horio, *J. Biochem.* **93**, 699 (1983).
- [10] K. R. Miller, *Nature* **300**, 53 (1982).
- [11] H. Engelhardt, W. Baumeister, and W. O. Saxton, *Arch. Microbiol.* **135**, 169 (1983).
- [12] W. Stark, W. Kühlbrandt, I. Wildhaber, E. Wehrli, and K. Mühlethaler, *EMBO J.* **3**, 777 (1984).
- [13] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, *J. Mol. Biol.* **180**, 385 (1984).
- [14] J. F. Imhoff, H. G. Trüper, and N. Pfennig, *Int. J. Syst. Bacteriol.* **1984**, 340.
- [15] J. F. Imhoff, *Int. J. Syst. Bacteriol.* **1984**, 338.
- [16] J. F. Imhoff and H. G. Trüper, *Arch. Microbiol.* **114**, 115 (1977).
- [17] R. Steiner and H. Scheer, *Biochim. Biophys. Acta* **807**, 278 (1985).
- [18] R. Steiner, E. Cmiel, and H. Scheer, *Z. Naturforsch.* **38c**, 748 (1983).
- [19] G. Feher and M. Y. Okamura, in: *The Photosynthetic Bacteria* (R. K. Clayton and W. R. Sistrom, eds.), p. 349, Plenum Press, New York 1978.
- [20] P. A. M. Michels and W. N. Konings, *Biochim. Biophys. Acta* **507**, 353 (1978).
- [21] K. Tanaka, T. Kakuno, J. Yamashita, and T. Horio, *J. Biochem.* **93**, 159 (1983).
- [22] U. K. Laemmli, *Nature* **227**, 680 (1970).
- [23] R. Theiler, F. Suter, H. Zuber, and R. J. Cogdell, *FEBS Lett.* **175**, 231 (1984).
- [24] G. H. Kaiser, J. Beck, J. U. von Schütz, and H. C. Wolf, *Biochim. Biophys. Acta* **634**, 153 (1981).
- [25] F. Jay, M. Lambillotte, and K. Mühlethaler, *Eur. J. Cell Biol.* **30**, 1 (1983).
- [26] R. A. Brunisholz, F. Jay, F. Suter, and H. Zuber, *Biol. Chem. Hoppe-Seyler* **366**, 87 (1985).
- [27] J. Peters and G. Drews, *J. Bacteriol.* **158**, 983 (1984).
- [28] G. Gimenez-Gallego, P. Suanzes, and J. M. Ramirez, *FEBS Lett.* **149**, 59 (1982).
- [29] V. Wiemken and R. Bachofen, *FEBS Lett.* **166**, 155 (1984).
- [30] R. A. Brunisholz, V. Wiemken, F. Suter, R. Bachofen, and H. Zuber, *Hoppe-Seyler's Z. Physiol. Chem.* **365**, 689 (1984).
- [31] R. A. Brunisholz, F. Suter, and H. Zuber, *Hoppe-Seyler's Z. Physiol. Chem.* **365**, 675 (1984).
- [32] R. J. Cogdell and H. Scheer, *Photochem. Photobiol.* **42**, 669 (1985).
- [33] H. J. M. Kramer, R. van Grondelle, C. N. Hunter, W. H. J. Westerhuis, and J. Amesz, *Biochim. Biophys. Acta* **765**, 156 (1984).

- [34] J. Ölze, *Biochim. Biophys. Acta* **509**, 450 (1978).
- [35] V. Wiemken, R. Theiler, and R. Bachofen, *J. Bioenerg. Biomembr.* **13**, 181 (1981).
- [36] R. L. Hall, P. F. Doorley, and R. A. Niederman, *Photochem. Photobiol.* **28**, 273 (1978).
- [37] F. Jay, M. Lambilotte, and F. Wyss, *Eur. J. Cell Biol.* **37**, 14 (1985).
- [38] P. A. Cuendet, H. Zürrer, M. Snozzi, and H. Zuber, *FEBS Lett.* **88**, 309 (1978).
- [39] G. E. Valkirs and G. Feher, *J. Cell Biol.* **95**, 179 (1982).